CYTOLOGICAL AND CYTOCHEMICAL STUDIES OF OOGENESIS OF POPILIUS DISJUNCTUS ILLIGER (COLEOPTERA–POLYPHAGA)

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The cytological events encountered in studies of gametogenesis pose many problems of interest to the cytologist. Although studies of spermatogenesis and oogenesis are of equal importance from the biological viewpoint, apparently much less attention has been given to the latter since the early part of the present century.

As pointed out by Wilson (1928), among the more important aspects of oogenesis are such problems as the morphological and physiological relations of the developing oocyte to the so-called accessory cells associated with it, the mechanism of oocyte growth and the formation of the yolk substance. The present paper is concerned mainly with the early development of the oocyte and its relation to the accessory cells of the ovary. Studies on the phases of oocyte growth and yolk formation are in progress and the results will be published elsewhere.

Broadly speaking, insect ovaries may be classified into two main types depending upon the presence or absence of so-called nurse cells. Thus the panoistic type (nutritive cells are lacking) is characteristic of the Orthoptera, Odonata, Isoptera and Aphaniptera while the meroistic type is characteristic of Coleoptera, Neuroptera, Hymenoptera and Hemiptera.

If the location of the nurse cells is taken into consideration, then the meroistic type may further be subdivided into the polytrophic condition, where nutritive or nurse cells alternate with the oocytes, and the telotrophic condition, in which case the nurse cells are confined to the apices of the ovarioles.

There is, however, some confusion in the literature with respect to the correct placing of families, especially in the case of the Coleoptera. Thus Imms (1948), following the classification of Stein (1847), considers the sub-order Polyphaga to be characterized by the telotrophic condition and the polytrophic condition to be characteristic of the sub-order Adephaga. On the other hand, Wigglesworth (1939) reverses the classification of Imms and Stein and in this respect appears to be following Weber (1933). Finally, Deegener (1928) cites the Coleoptera-Polyphaga as possessing the telotrophic type of ovary.

Hence, according to Imms, *Popilius disjunctus* should possess ovaries of the telotrophic type, while according to Weber and Wigglesworth they should possess polytrophic ovaries. More recently, the structure of the ovariole was investigated by Krause (1946) who concluded that the ovarioles were of the typical telotrophic type. The cytological observations reported below support the conclusion that the ovarioles of *Popilius disjunctus* exhibit a modified telotrophic condition.

The relationship between nurse cells and oocytes has been investigated by many workers; see for instance the papers of Giardina, Korschelt, Nussbaum-Hilarowicz

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(reviewed in detail by Wilson, 1928), Shaffer (1920) and others. Such investigations suggested that nurse cells were abortive ova specialized for the formation of nutritive materials to supply the needs of the developing egg. This problem was re-investigated in the Hemiptera by Schrader and Leuchtenberger (1952) using cytochemical procedures. Their findings showed that the nurse cells did contribute material to the egg cytoplasm and furthermore that this nutritive substance was in part derived from the desoxyribose nucleic acid (DNA) of the nurse cell nuclei.

Materials and Methods

Specimens of *Popilius disjunctus* Illiger (Passalidae) were obtained from a biological supply house as required. In addition a number of *Macrodactylus subspinosus* Fabricius (Scarabaeidae) were collected in Newton, Massachusetts, and used to supplement the studies on *Popilius disjunctus* material. Both families are members of the superfamily Lamellicornia.

A. *Fixation*

The gonads were dissected free of tracheoles and fixed in Carnoy's solution (3:1) for two hours. The tissues were washed several times in absolute alcohol, cleared in benzene and embedded in 56–58° C. Tissuemat. Sections were cut at 10 μ and at 5 μ in order to provide sections containing whole nuclei and thin sections of nuclei for subjection to various cytochemical procedures. It should be pointed out that the dissections were performed immediately or within three days of receipt of each shipment.

B. *Staining*

1. *Nucleic acids*

a. The Feulgen reaction. The Feulgen reagent was prepared according to the method of Stowell (1945) and sections were stained for two hours at room temperature following optimal hydrolysis (12 minutes) in 1 N HCl at 60° C. All slides were stained in the same batch of reagent though at different times.

b. Methyl green staining. The dye was first purified by shaking a 2% aqueous solution with chloroform until the washings became colorless. A calculated aliquot of the purified dye solution was added to the phenol-glycerin mixture as used by Pollister and Leuchtenberger (1949) to give a final dye concentration of 0.2%. It has been shown by Pollister and Leuchtenberger (1949) and by Alfert (1952) that pretreatment of the slides with hot reagents causes a lowering of the amount of methyl green bound by nuclei. Therefore the staining reaction was carried out at 37° C. for 1½ hours. Slides were then rinsed in ice-cold water, blotted and rinsed in tertiary butyl alcohol and differentiated overnight in fresh tertiary butyl alcohol. This procedure was carried out in order to insure chemical staining (Michaelis, 1947).

c. Azure B. Sections were stained in 0.2% watery solution of azure B at pH 4.0 for 3 hours at 40° C., according to the method of Flax and Himes (1952). After rinsing, the slides were differentiated in the same manner as for methyl green.

Sections were also stained by the methyl green and azure procedures following treatment with ribonuclease (see below).

d. Toluidine Blue O. Preliminary investigations of basic staining were carried out by staining sections for 15 minutes in a 0.25% aqueous solution of toluidine blue followed by a short differentiation in absolute alcohol.

2. *Proteins*

a. Millon reaction. The reagents were prepared and used as described by Pollister (1950); see also Bryan (1951).

b. Fast green. A 0.1% solution of fast green was prepared and used as described by Schrader and Leuchtenberger (1950) and by Bryan (1951).

3. *Additional techniques*

a. Ribonuclease. Ribonuclease was obtained from the Worthington Biochemical Sales Company. The enzyme was boiled for five minutes in a few drops of saturated ammonium sulphate solution in order to remove any proteolytic impurities. The resulting solution was then diluted with redistilled water to give a final enzyme concentration of 0.2 mgm. per ml. Slides were exposed to the enzyme solution for four hours at 45° C. Control slides were immersed in ammonium sulphate solution (0.2 mgm. per ml.) but at the same temperature and for the same amount of time as the test slides.

b. Periodic acid-Schiff reaction (P.A.S.). The reagents were prepared according to McManus (1948). Sections were oxidized with periodic acid (0.5%) for 5 minutes, rinsed in distilled water and stained in leucobasic fuchsin solution for 15 minutes. After staining in the leucobasic fuchsin solution the slides were passed through a bleach bath (3 changes of 10 minutes each) consisting of 10 ml. of 1 N HCl, 10 ml. of a 10% $K_2S_2O_5$ solution and 180 ml. of distilled water.

Following this treatment the slides were washed in water, dehydrated and mounted. In certain cases methyl green was used as a counterstain.

4. *Photometry*

Measurements of the amount of dye bound or the color produced in the tissue sections were made with an apparatus essentially the same as that described by Pollister and Ris (1947) and by Bryan (1951). However, instead of a Weston micro-ammeter, a General Electric micro-ammeter having a sensitivity of 0.0014 micro-ampere per millimeter was used to measure the photomultiplier output. By means of a calibrated Ayrton shunt (total resistance 10,000 ohms) the sensitivity level of the meter was adjusted to the optimal range for recording the output of the photomultiplier tube. Interference filters were used to isolate from the spectrum of an AH_4 mercury lamp the spectral lines used in making photometric measurements. Each line isolated was close to the absorption maximum of the color reaction concerned.

It was necessary to compare data obtained from slides prepared at different times. Therefore, in order to check on the reproducibility of the reactions used, sections from one ovary were mounted on each slide and used as a standard. The extinction value of the blank prepared for the Feulgen reaction was found to be negligible.

5. *Computation*

The transmittance data were obtained by the "plug" method described by Ris and Mirsky (1949) and by Swift (1950). In addition, whenever warranted, the

mean optical path (MOP) of each nucleus was determined and used in the computations as described by Bryan (1951). The transmittance data were converted into extinction values ($\log_{10}$ 1/T) and the extinction data were computed as described by Bryan (1951).

It is self-evident that the amounts of substance computed from extinction data are expressed in arbitrary units which are, however, related to the absolute amounts of absorbing substance present.

Results

A. *Cytological Studies*

Since there is in the literature some confusion with respect to the classification of the Coleoptera, based on the structure of the ovariole, the cytological findings are reported in some detail.

In *Popilius disjunctus* the typical number of ovarioles is two per ovary while in *Macrodactylus subspinosus* it is six. The histological structure is essentially similar in both species. Unless otherwise indicated, the following observations are based on studies of *Popilius* material.

Histologically speaking, each ovariole may be divided into two major regions—the germarium and the vitellarium. A longitudinal section of an ovariole is depicted in Figure 1.

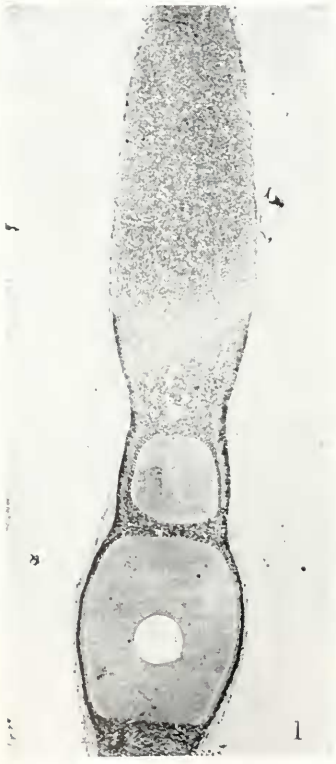
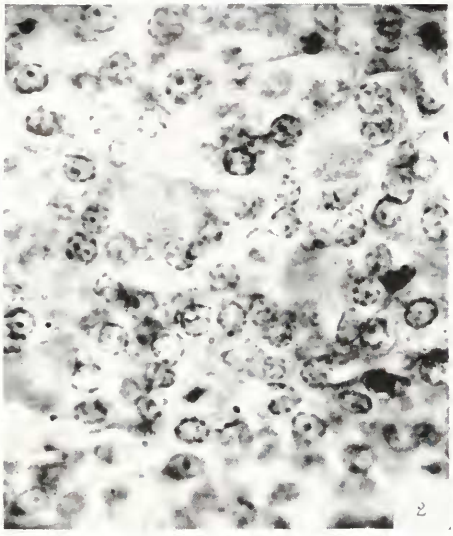
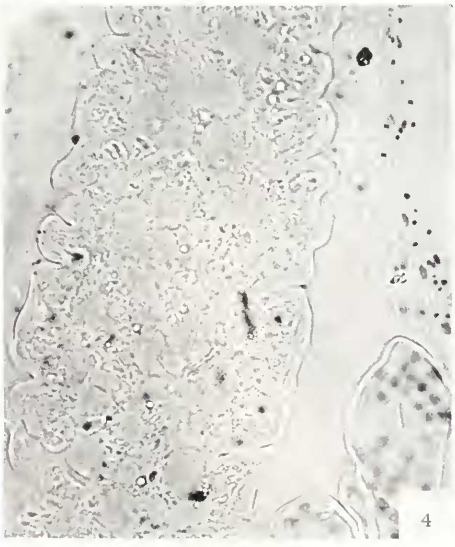


Figure 1. Longitudinal section through an ovariole from a mature adult. Stained with azure B. Photo 100 ×.

The germarium

At the apex of each ovariole the germarium becomes drawn out into an attenuated filament in the form of a spiral. As may be seen from Figure 2, this region in the immature female presents a distinctly different appearance from the





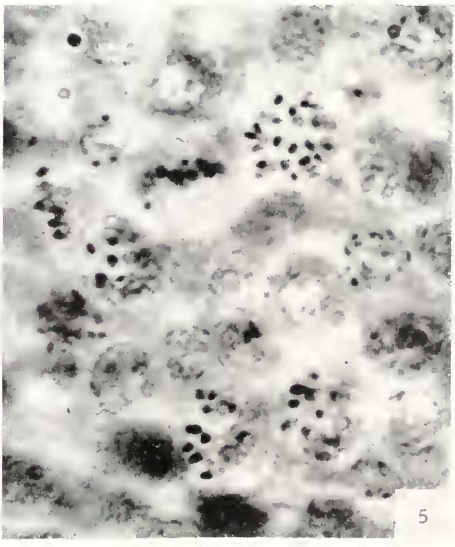


FIGURE 2. Spiral tip of germarium of an immature adult. Note the masses of acidophilic material. Feulgen counter-stained with fast green. Photo 1200 ×.

FIGURE 3. Similar region of germarium but from an individual a few weeks older than in the case of Figure 2. Stained with azure B. Photo 600 ×.

FIGURE 4. Spiral tip of germarium from a breeding female. Feulgen stain. Photo 400 ×.

FIGURE 5. Oogonial mitoses in germarium of immature adult. Feulgen counter-stained with fast green. Photo 3000 ×.

rest of the germarium. The cells appear to surround large masses of material which stains strongly with acid dyes as well as giving a positive Schiff (P.A.S.) reaction.

In older individuals the nuclei are slightly smaller but the chromatin is much more homogeneous. This condition is depicted in Figure 3. The apical region

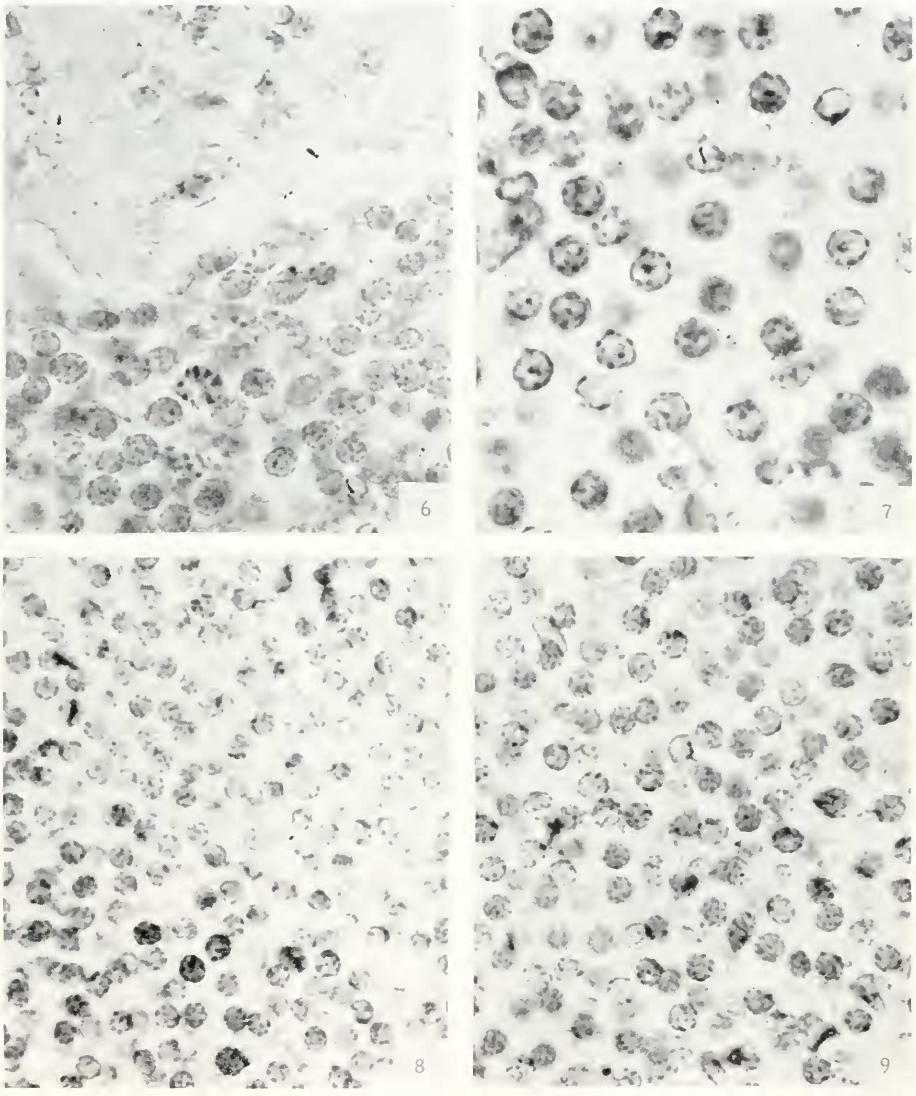


FIGURE 6. The junction of the spiral tip with the body of the germarium. Feulgen. 1200 ×.

FIGURE 7. Nuclei from the body of the germarium of a young adult, showing partially condensed chromosomes. Feulgen stain. Photo 1700 ×.

FIGURE 8. Distal portion of germarium of a young adult. Feulgen stain. Photo 1000 ×.

FIGURE 9. Proximal portion of germarium from same ovariole as in the preceding figure. Feulgen stain. Photo 1000 ×.

of an ovariole taken from a laying female is illustrated in Figure 4. It is readily seen that the apical region is now almost devoid of nuclei. There is no evidence that this condition is brought about by pycnotic degeneration of the nuclei.

In the body of the germarium the cells are small and present a closely packed appearance; the nuclei are spherical and there is but little cytoplasm. Mitotic figures are abundant in this region of the ovariole of sexually immature females (Fig. 5). Counts of oogonial metaphase plates indicate the diploid number of chromosomes to be twenty-six, as previously reported by Shaffer (1917) and by Smith (1953). On the other hand, examination of ovarioles of mature females indicated mitotic figures to be absent from the germarium.

Close to the spiral tip, most of the nuclei exhibit a typical interphase condition (Fig. 6); however, nucleoli are extremely small or absent. More posteriorly, in young females, most nuclei appear to have been arrested in late prophase, the chromosomes being partially condensed and peripherally located (Fig. 7). Here, in sections stained by the Feulgen technique followed by counterstaining with fast green, there may be seen small globules or, occasionally, thread-like bodies which stain with the acid dye. This acidophilic material is usually located in the central region of the nucleus.

While the nuclei at any one level in the germarium show a uniformity of size, those in the proximal part show an increase in size such that the average nuclear volume is twice that of the younger (more distal) nuclei—compare Figures 8 and 9.

In the sexually mature female, the oocytes adnate to the vitellarium appear to be undergoing meiosis (Fig. 10). There is no indication that development progresses beyond the diplotene-diakinesis stage. Instead, the chromosomes become diffuse and cannot be identified clearly. It is at this time that the oocyte enters the growth phase.

The vitellarium

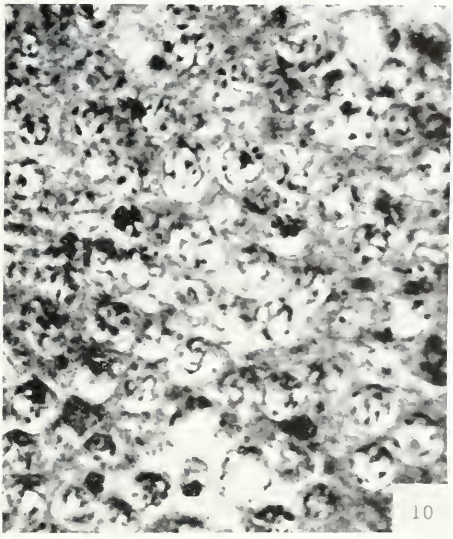
The zone of transition between the germarium and the vitellarium is depicted in Figure 11. Here the enlarging oocytes appear to be embedded in layers of cells greatly elongated in a direction perpendicular to the long axis of the ovariole.

As the oocytes pass through this region they often develop cytoplasmic finger-like projections (Fig. 11). These processes cannot be traced beyond the distal border of the transitional zone. It would appear that they may be produced in response to the squeezing effect of the surrounding cells on the oocyte cytoplasm as the egg cells move through this zone into the body of the vitellarium.

In the body of the vitellarium each oocyte can be seen to have acquired a sheath of follicle cells. In the case of the younger oocytes, the follicle is several cell layers thick whilst in older oocytes it has been reduced to a single layer of cells.

As the oocyte enters the growth phase there is a marked increase in volume of nucleus and cytoplasm. As the nucleus increases in size, there is a corresponding decrease in staining intensity; the mature nucleus is Feulgen-negative and does not stain with basic dyes. There is no evidence of marked nucleolar activity during the oocyte growth phase. If, however, protein stains are used, then it can be seen that the nucleus stains darkly at all times. As can be seen from Figure 11 the nucleus at first has a granular appearance. In older eggs, the protein stain is distributed quite homogeneously throughout the nucleus.

Cytological examination of the germarium (including the spiral tip) failed to reveal any signs of extensive cellular degeneration. No indications were found of the existence of plasmatic strands connecting the germarium with the developing eggs. In this respect the ovaries of *Popilius* differ markedly from the condition



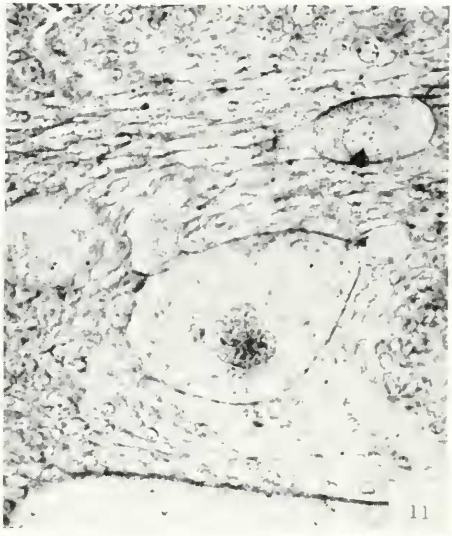


FIGURE 10. Portion of germarium adnate to the vitellarium, showing meiotic figures. Feulgen counter-stained with fast green. Photo 1500 ×.

FIGURE 11. The zone of transition between the germarium and vitellarium, showing an oocyte with cytoplasmic processes. Stained with the bromphenol blue-mercury reagent. Photo 500 ×.

FIGURE 12. Epithelium of the lateral oviduct, showing polyploid nuclei; compare with Figure 13. Feulgen stain. Photo 1200 ×.

FIGURE 13. Oviduct epithelium from the same section as figure 12. Note the clumps of heterochromatin. Feulgen stain. Photo 1200 ×.

found in the Hemiptera. Subsequent examination of the *Macroactylus* material agrees with the observation made on the *Popilius* material.

In the vitellarium the eggs are arranged in a single row. Posterior to the oldest egg the ovariole is closed by a plug of epithelial cells which is ruptured at the commencement of oviposition.

Eggs from each pair of ovarioles are released into the lateral oviducts which unite to form the duct leading to the exterior. The nuclei of the cells lining these ducts exhibit several interesting phenomena. Here nuclear volumes fall into several classes simulating a polyploid series. While the smaller nuclei tend to be homogeneous (Fig. 12), the larger nuclei have a markedly different appearance (Fig. 13). Many strands of chromatin may be seen and, in most cases, they can be traced to clumps of heterochromatin located close to the inner surface of the nuclear membrane. Counts of these clumps, while somewhat variable, correspond closely to the diploid number of chromosomes. The amount of chromatin in each nucleus would appear to be several times as great as that found in the nuclei of other tissues.

It is obvious from the foregoing observations that the structure of the *Popilius* ovariole is quite different from that encountered in the Hemiptera and in other insects possessing the telotrophic type of ovariole.

B. *Cytochemical findings*

1. *DNA-Feulgen*

The data obtained are summarized in Table I. The values represent the mean extinctions, volumes and amounts of regenerated Feulgen dye per nucleus. In certain cases a few nuclei in the sample measured were small enough to warrant consideration of the MOP (see above) when computing amounts of DNA-Feulgen. Such values are, in the following table, indicated by a dagger (†) in the last column.

Measurements were also made on spermatid nuclei in order to provide data to be used as a standard, since the spermatid nucleus contains the haploid amount of DNA. These data are presented in Table I.

Cytological examination of the follicle cell nuclei showed the chromatin to be rather irregularly distributed. In addition, nuclei of follicle cells of older eggs are extremely large and show different degrees of flattening. For these reasons no photometric data pertaining to follicle cell nuclei are presented in this report.

2. *Methyl green*

In the present work it was repeatedly observed that the cytoplasm of oogonia, oocytes and follicle cells was stained by methyl green. In the case of the cells comprising the body of the germarium strong staining of the cytoplasm was observed; whereas the developing oocytes in the vitellarium, together with the investing follicle cells, evinced much weaker reactions. Overnight pretreatment of slides in the cold with dilute perchloric acid (Ogur and Rosen, 1950) abolished cytoplasmic staining. Complete suppression of cytoplasmic staining was achieved also by the use of the enzyme ribonuclease (see above).

TABLE I

DNA-Feulgen content of nuclei

Tissue and stage	Class	No. of nuclei measured	Mean extinction E_{549}	Mean nuclear volume microns^3	Mean amounts in arbitrary units
Testis-spermatid	I (n)	21	0.041	40.8	0.53±0.03*
Ovary: spiral tip of germarium					
(a) Immature adult	II (2n)	8	0.096	54	1.09±0.09
II→III	II *a*	24	0.132	60.5	1.60±0.02
	III (4n)	10	0.170	58.6	2.14±0.08
(b) Mature adult	II (2n)	16	0.190	43.1	1.12±0.03*
II→III	II *a*	13	0.143	44	1.46±0.03*
	III (4n)	2	0.162	43.1	2.05
Body of germarium					
(a) Immature adult Oogonia	II (2n)	19	0.086	70.4	1.11±0.04
II→III	II *a*	20	0.146	58.4	1.73±0.02
	III (4n)	11	0.191	64.6	2.42±0.08
(b) Mature adult oocytes—distal portion of germarium	III (4n)	20	0.188	58.4	2.27±0.09
III→IV	III *a*	2	0.232	68.5	3.13
	IV (8n)	3	0.350	52.8	4.96
Oocytes—proximal portion of germarium	II (2n)	3	0.090	52.8	1.35
III→IV	III (4n)	23	0.126	106	2.10±0.09
	III *a*	1	0.144	124	3.09
Epithelium of lateral oviduct	II (2n)	3	0.226	17.5	1.23
	III (4n)	15	0.269	30.4	2.06±0.13
	IV (8n)	27	0.461	39.9	4.31±0.14*
	V (16n)	29	0.641	72.5	8.86±0.18

* MOP considered.

3. *Azure B*

When used as described by Flax and Himes (1952), azure B stains DNA orthochromatically blue-green and RNA metachromatically purple. As is the case with other basic dyes, azure B failed to stain the nuclei of oocytes contained within the vitellarium. The pattern of cytoplasmic staining, obtained with azure B, is essentially the same as that obtained with methyl green. The staining of the cytoplasm was completely abolished by a prior digestion of the slides with ribonuclease.

4. *Protein methods*

It was found that the masses of acellular material in the spiral tip of the germarium stained quite strongly with fast green and less intensely with the Millon reagent.

In very young oocytes, before the growth phase has commenced, the threads of chromatin stain quite strongly while the cytoplasm is only weakly stained. In later

oocytes, the nucleus presents a more homogeneous picture and, in contrast to the results obtained with basic dyes, still stains in an intense manner. In such nuclei the individual chromosomes can no longer be identified. As stated earlier, the nucleolar system does not show signs of intense or prolonged activity; only in a few older egg cells have small intranuclear bodies been observed. Studies on the protein content of oocytes are still going on and the results will be published at a later date.

5. *The periodic acid-Schiff reaction*

It was found that in the spiral tip of the germarium the acellular material which stained strongly with fast green also gave a strongly positive reaction with the Schiff reagent. In addition, small amounts of Schiff-positive material were distributed throughout the body of the germarium in an irregular fashion. In the cytoplasm of intermediate oocytes may be seen Schiff-positive droplets and granules. These structures are peripherally located, lying just below the surface of the egg. They are, therefore, in close proximity to the inner surface of the surrounding follicle cells. In much older eggs, where the chorion is present, the cytoplasmic Schiff-positive material is more uniformly distributed. It is also of interest that the tunica propria and the chorion give a strongly positive Schiff reaction.

Discussion

1. *DNA-Feulgen*

The germarium. From the data summarized in Table I it may be inferred that, in the immature female, the body of the germarium is essentially filled with oogonial cells multiplying rapidly by mitosis. The data indicate that the oogonial nuclei build up their DNA content to four times the haploid value prior to division—at which time the amount falls to that of the diploid value. Similar findings, in various animal tissues, have previously been reported by Swift (1950) and others.

The apical region of the germarium presents a similar picture of mitotic activity. Often strands of cells from the apical region appear to interdigitate with the most anterior layers of oogonial cells contained within the body of the germarium. The close correspondence of the photometric data, together with the cytological observations, suggests that the type of nucleus found in each region may be, in fact, identical.

In the case of the mature female the spiral tip, though mitotic figures were absent, still contains nuclei which possess an amount of DNA in excess of the diploid value. As reference to Table I shows, only two nuclei were found with the Class III amount of DNA; whereas Class II *a* (intermediate) and Class II nuclei were found in approximately equal numbers. The data show that mitotic activity continues in this region longer than in the body of the germarium. Perhaps the lack of Class III nuclei is an indication that mitosis has, by this time, slowed down or ceased.

According to Krause (1947), early in development a small group of primordial germ cells forms a knob-like structure at the distal tip of each arm of the Y-shaped gonad anlage. Later this knob gives rise to the spiral tip of the germarium. The central and basal portions of each anlage become hollowed out to form the cylindrical egg tube. This result is achieved by extensive cellular degeneration. Such degenerative changes were not reported to occur in the future spiral tip of the germarium.

Hence it may well be that most, if not all, of the functional primordial germ cells are separated from the rest of the anlage very early in development. Then it would follow that their derivatives, the definitive oogonia, as they are formed, pass into the body of the germarium where they multiply still further.

It is also possible that the primordial sex cells contained within the knob-like tip of the anlage are destined to become trophocytes or nurse cells. Such an interpretation would not be necessarily in conflict with the photometric data. It would be expected that such cells should undergo mitosis, during the time that oogonial divisions were taking place, in order to supply adequately the later nutritional needs of the oocytes.

On purely structural grounds, however, it would appear that such an interpretation should not be given undue emphasis for it is obvious that any nutritive material, in order to reach the growing egg cells, must diffuse through the entire body of the germarium.

At the present time it is not possible to discount fully the latter alternative. Though the presence of large amounts of PAS-positive substance in the apex of the germarium is suggestive of a trophic function for this region, it is clear that the apical zone cannot play as important a role in this respect, as does the end zone in the Hemiptera (see Schrader and Leuchtenberger, 1952). Such a conclusion is further supported by the absence of plasmatic strands connecting the apical region with the developing oocytes.

With respect to the DNA content, the condition prevailing in the body of the germarium of mature females is quite different. The data indicate that, with few exceptions, all the nuclei measured now have the Class III amount of DNA.

In the case of the proximal region three nuclei, out of a sample of twenty-seven, were found to possess the diploid amount of DNA. It is possible that these nuclei were the products of recent mitotic divisions and that the DNA content had not yet been built up to the final amount. It is also possible that these nuclei may be early follicle cell nuclei which were measured in error although this does not seem very probable for cytological reasons.

It was also observed that occasionally nuclei were encountered which were stained much more darkly than the typical oocyte nucleus, but which were of about the same size. In these observations perhaps lies the explanation for the finding, in the germarium, of nuclei possessing more than the tetraploid amount of DNA. It would appear likely that such nuclei, present in small numbers, are abnormal and do not give rise to mature eggs.

The vitellarium. The chromatin of the nuclei of oocytes in the zone of transition is distributed in an irregular manner. For this reason it was not possible to obtain valid photometric data.

It is well known that as the oocyte nucleus increases in volume, the intensity of the Feulgen stain and the degree of basophilia diminish. Nuclei of the developing oocytes in the vitellarium do not stain with basic dyes and also appear to be Feulgen-negative. Results such as these have often been interpreted to mean that there is no DNA in the nuclei of oocytes during the phase of growth. It is more probable that the decrease in intensity of the Feulgen reaction is attributable to a "diluting effect" of the increasing amounts of protein in the egg cell nucleus. This problem has been considered in more detail by Alfert (1950), and further remarks need not be made here.

As pointed out earlier, the follicle cell nuclei are not well suited to photometric needs. It is true that in the case of the younger follicles the nuclei are smaller and the chromatin is fairly homogeneously distributed. However, in these cases it was found that there was considerable overlapping of nuclei in the sections prepared. The degree of overlapping was such as to preclude the carrying out of photometric studies. It is of interest that, unlike the conditions obtaining in other insects, the cells of the more mature follicles do not appear to be multinucleate. Instead the volume of each nucleus has undergone a marked increase for it was found that the volumes of these nuclei were of the order of 1000 μ^3.

The lateral oviducts. Photometric measurements of DNA-Feulgen were made on nuclei of the epithelial lining of the lateral oviducts. The data are presented, in summary form, in Table I. It should be pointed out that only nuclei in which the chromatin was homogeneously distributed were chosen for measurement; no attempts were made to obtain data from nuclei such as those depicted in Figure 13.

It may be seen that the epithelial cell nuclei appear to contain amounts of DNA in multiples of the Class II (diploid) amount. It is also evident that nuclei containing the Class II amount are very infrequently met with, while nuclei possessing the Class IV or Class V amount of DNA constitute the most frequent classes.

The data suggest that there is a tendency for an increase in DNA content to be accompanied by an increase in nuclear volume. Whether or not a similar change in protein content occurs is unknown at the present time. Examination of the tissue involved failed to reveal any indication of metaphase plates or other phases of division. It is, therefore, quite probable that the mechanism underlying the formation of such nuclei, possessing amounts of DNA in multiples of the diploid amount, may be one of endomitosis. That the epithelial cell nuclei are endopolyploid in nature is further supported by the observations presented in the cytological section of this report.

This occurrence of polyploid cells in insect tissues is not unique for, as is well known, endopolyploid nuclei have been encountered in a variety of insects. Briefly, they have been reported by Geitler (1937) to occur in various tissues of *Gerris lateralis* and similar findings have been described by Berger (1938) with respect to the larval intestine of *Culex pipiens*. More recently, White (1951) has shown endopolyploid nuclei to be present in the testicular envelope of certain grasshoppers.

It is not possible at the present time to attach any functional significance to the present findings. Examination of the cytoplasm of these cells precluded the idea that the epithelial tissue may possess a glandular function. The existence of polyploid nuclei in the lateral oviduct tissue is perhaps a cytological indication of ageing.

2. *General considerations*

The basic dyes, azure B and toluidine blue, were used in conjunction with ribonuclease to study the cytoplasmic basophilia of oocytes and oogonia. During the course of these studies it was noted that the acellular material in the apex of the germarium stained, at best, very faintly with the dyes used. These observations indicate that the acellular material does not contain large amounts of RNA.

It would appear to be of some significance that the cytoplasmic basophilia of the follicle was found to be particularly intense during the periods of oocyte growth and chorion formation. It follows, therefore, that the follicle may be instrumental in

supplying the growing egg with nutritive material. It would also appear that follicle cells are intimately concerned with the formation of the chorion.

It is a striking fact that in *Popilius*, nucleoli are never very prominent components of the nucleus. Perhaps the variable form of the nucleoli may be attributed to the absence of "effective" nucleolar organizing regions. Such a conclusion would not be in conflict with the observations since Heitz (1931) has reported the formation of nucleoli despite the fact that nucleolar regions were lacking.

That the nucleolar system, as envisaged by the Caspersson school, does not achieve prominence during the period in which oocytes are rapidly increasing in size may argue against the idea that, in *Popilius*, nucleoli play an important role in the growth process. At the present time, however, the mechanism of oocyte growth must remain an open question.

The life history of *Popilius* has been studied by Riley (1872), Wheeler (1923), Gray (1946) and others. Oviposition commences during May and continues until early July, the peak of activity being in June. According to Gray (1946), relatively few eggs are laid by any one female. The average number appears to be of the order of thirty-five and sixty appears to be the maximum.

From mid-August until late in October immature red-colored adults are found in the colonies; later than this, all individuals appear to be the black-colored mature adults. It is of interest that in the present work, no beetles taken from the colonies later than August proved to be the mature adults of the previous spring, indicating that death must occur shortly after the end of the reproductive phase.

Examination of the ovarioles from immature adults taken in late September revealed the presence of developing eggs in the vitellarium. Ovarioles of immature individuals taken a few weeks earlier proved to contain numerous oocytes undergoing meiosis. The stages found ranged from zygotene to diakinesis. It should be stated that the fixative used (Carnoy 3:1) does not preserve the meiotic figures well enough to permit identification of the stages with absolute certainty.

From the foregoing observations it would appear that the first eggs laid must have been present in the vitellarium for some months prior to the breeding season. However, this does not seem to be the case, for it was repeatedly observed that in individuals taken over the winter months, the oldest eggs appeared to be undergoing degeneration and resorption. In one case, the posterior part of the vitellarium had been invaded by phagocytes, suggesting that phagocytosis may be an important factor in the resorptive process. It would appear then that during the winter months mature females may actually rely upon the almost mature eggs and their follicles as a source of supplementary nutritive material. Individuals obtained a few weeks prior to the commencement of the breeding season failed to reveal any indication of degenerative processes in the vitellarium.

That unfavorable environmental conditions may be responsible for the degenerative process is perhaps further suggested by the following observations. Ovarioles taken from *breeding* females immediately upon receipt appear to be quite normal whereas indications of oocyte degeneration are apparent if such females are kept under laboratory conditions for longer than ten days. In addition, there are the observations of Gray (1946) to the effect that under laboratory conditions females do not lay for more than six or seven days and, furthermore, the last eggs to be laid under such conditions are usually abnormal in appearance.

Summary

1. The cytology of the ovarioles of *Popilius*, together with their associated ducts, is described.

2. Cytological and cytochemical studies of the germarium suggest that the ovariole constitutes a modified telotrophic type.

3. Microspectrophotometric measurements of nuclei, stained by the Feulgen reaction, indicate that during the interphase period oogonial nuclei build up their DNA content to twice the diploid amount and that after division the daughter nuclei each contain the diploid amount. The data also indicate that in mature females primary oocyte nuclei contain the tetraploid amount of DNA.

4. Cytological observations, together with photometric measurements, suggest that the epithelium of the lateral oviducts is an endopolyploid tissue.

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